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A SPECTROPHOTOMETRIC METHOD FOR BROMSULFALEIN DETERMINATION IN NORMAL JAUNDICED AND LIPEMIC SERUM*

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The bromsulfalein test for liver function which is widely used has certain drawbacks in estimating the amount of dye retained. The Rosenthal Comparator Block[1] method is simple and accurate unless an excessive amount of bile or fat is present in the serum. The photoelectric method of Gaebler[2] is accurate but also time consuming. The problem which presented itself was to find a method of extraction of the dye which would be simple, give complete recovery and yet eliminate the interfering fat or pigment.

It was decided to explore the possibility of extraction by the Kingsley[3] method of separation of albumin and globulin as used in the biuret method of protein determinations.

To serum collected forty-five minutes after the injection of dye, a dilution was made by adding 1 ml. serum in a centrifuge tube to 9 ml. of 23% sodium sulfate solution to which a small amount of 10% sodium hydroxide had been added. After subsequent shaking with ether and centrifugation, it was noted the alkaline, sodium-sulfate albumin phase was tinted purple by the retained dye. That none of the dye was retained by the globulin precipitate was evident when, upon addition of sodium hydroxide no purple color developed. None of the dye was extracted by the ether.

A curve was prepared for the Coleman Junior Spectrophotometer using pooled, clear, unhemolyzed serum to which was added bromsulfalein dye to make the standard. When mixed in vitro the dye combined with the serum and could be separated by shaking with the alkaline sodium sulfate and ether. A portion of the pooled serum was saved for dilutions of the serum dye solution in preparation of the curve.

* First A.S.M.T. Award paper read Annual Convention, Louisville, Ky., June, 1953.

A series of one hundred tests was run by both the Comparator Block Method and the new technic, reading results on the spectrophotometer.

The following list of cases shows the comparison of results by the two methods:

Case Number	Comparator Block	Spectrophotometer
1	Less than 5%	1%
2	30%	31%
3	20%	17%
4	40%	36%
5	10%	12%
6	5%	6%
7	Less than 5%	2%
8	60%	56%
9	20%	20%
10 icteric serum	40%—50%	43%
11 icteric serum	40%—50%	45%
12 icteric serum	20%—30%	20%
13 icteric serum	40%—50%	30%
14 icteric serum	15%	13%
15	0	1%
16	8%	7%
17	0	0
18	20%	21%
19	Less than 5%	4%
20	20% to 30%	24%

Comparison shows close relationship on lower values but a clear-cut reading cannot be made on sera showing jaundice or higher dye retention by the Comparator Block method.

The tests were all run on patients in the fasting state using the 5 mgs. of bromsulfalein per kilogram of body weight. In all cases the 5% sterile bromsulfalein solution obtained from Hynson, Westcott and Dunning (Baltimore, Maryland) was injected and blood withdrawn from the opposite arm forty-five minutes after the injection.

Normal values with this method show 0 to 5% of dye retained at 45 minutes.

Principle

Blood serum drawn 45 minutes after the injection of bromsulfalein dye is treated with alkalinized 23% sodium sulfate. Utilizing the Kingsley principle of the separation of albumin and globulin, the globulin is removed by centrifugation and the dye-tinted albumin-sodium-sulfate phase is read in the spectrophotometer against a calibration curve which has been prepared from pooled serum to which bromsulfalein dye has been added. Calibration is based on administration of 5 mgs. of bromsulfalein per kilogram of body weight.

Determination

Patient should be in fasting state.

Five mgs. of bromsulfalein per kilogram of body weight are injected intravenously. Forty-five minutes after injection blood is withdrawn from the opposite arm, care being taken to avoid hemolysis. The blood is allowed to coagulate and the serum removed.

With this technic, sera containing excessive amounts of fats and biliary pigments do not interfere with the accuracy of the results, but hemoglobin will do so at the wavelength used.

Test Procedure

Apparatus

(1) Ostwald-Folin pipettes 1 ml.
(2) Serological pipettes 1 ml. graduated in 0.01
(3) Serological pipettes 0.2 ml. graduated in 0.01
(4) Serological pipettes 10.0 ml. graduated in 0.1
(5) Volumetric flasks 50 and 100 ml.
(6) 15 ml. graduated centrifuge tubes
(7) Test tubes 15 x 100 mm. for collecting blood samples.

Reagents

(1) Sterile bromsulfalein dye (50 mgs. per cc. from Hynson, Westcott and Dunning, Baltimore, Maryland).
(2) Ethyl ether—Squibbs anesthesia.
(3) 100% dye standard for preparation of calibration graph: Place 10 mgs. bromsulfalein in 100 ml. volumetric flask using the sterile dye solution containing 50 mg. per cc. 10 mgs. is represented by 0.2 cc. of bromsulfalein solution. dilute to mark with normal clear, unhemolyzed pooled serum. Mix thoroughly.
(4) 23% sodium sulfate. Weigh 23 gms. anhydrous sodium sulfate, heat with about 60 cc. distilled water. Cool to 37° C., transfer quantitatively to 100 ml. volumetric flask. Dilute to volume at 37° C. Store in incubator at 37°C.
(5) Alkaline 23% sodium sulfate. Alkalinize quantity necessary for test by adding 0.5 ml. of 10% sodium hydroxide to each 50 ml. of 23% sodium sulfate.

Technic

(1) In 15 ml. graduated centrifuge tube place 0.5 ml. clear unhemolyzed serum from 45 minute blood specimen.
(2) Add 4.5 ml. alkalinized 23% sodium sulfate. Mix.
(3) Add 2 ml. ethyl ether. Cover tube with thumb covered with finger-cot, shake for 30 seconds.
(4) Centrifuge 1500 R.P.M. for 5 minutes.
(5) Tip the tube and pipette the sodium sulfate layer below the ether and globulin precipitate.
(6) Transfer to 10 x 75 mm. Cuvette.
(7) Read in Coleman Junior Spectrophotometer at 575 mu wave

length against reference blank of alkalinized sodium sulfate at T = 100.

(8) Read result in % from calibration chart.

(9) Blank Control Tube—Set up a second 15 ml. graduated tube with 0.5 ml. serum and 4.5 ml. 23% sodium sulfate to which no sodium hydroxide has been added. Shake with ether. Centrifuge and read the sodium sulfate phase in the spectrophotometer using the sodium sulfate reference. This control will take care of bile or slight amount of pigment.

Test — Blank = bromsulfalein %.

Note: The bile color does not show an appreciable change at this wave length, however, extracted pigment from hemolyzed cells will do so.

Preparation of Calibration Graph

100% Dye Standard (Reagent # 3)

Make dilutions from the 100% dye standard using pooled dye-free, clear serum as diluent according to following table:[4]

Dye Percent	Stock (Dye Plus Serum)	Dye-Free Serum
5	0.25 ml.	4.75 ml.
15	0.75 ml.	4.25 ml.
30	1.50 ml.	3.50 ml.
50	2.50 ml.	2.50 ml.
70	3.50 ml.	1.50 ml.
100	5.00 ml.	None

Plot curve using dilutions as follows:

In each of six 15 ml. graduated centrifuge tubes place 1 ml. of above dilutions.

Add 9 ml. alkalinized 23% sodium sulfate (reagent No. 4).

Mix—add 2 ml. ether. Shake 30 seconds.

Centrifuge 5 min. 1500 R.P.M.

Read the dye-tinted sodium sulfate layer in Coleman Junior spectrophotometer with reference T = 100 at 575 mu wave length and 10 x 75 mm. Cuvette.

Reference solution = 23% alkalinized sodium sulfate.

Plot curve on semilog graph paper. Points fall on a straight line passing through the origin.

Summary

It is felt the proposed technic is of value in estimating the amount of dye retention in normal jaundiced and lipemic serum and furnishes an accurate and satisfactory method for extraction. The technic also opens the possibility of a means of separation and determination of other kinds of injected dyes.

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AGGLUTINATION OF ERYTHROCYTES IN HAYEM'S SOLUTION—A REPORT OF TWO CASES*

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Agglutination of erythrocytes in Hayem's solution is a phenomenon which has been observed for a number of years. De Angelis and Huntsinger[3] thought it to be a grave prognostic sign in a number of pathologic conditions but later De Angelis and De Angelis[2] modified this concept to that of an aid in prognosis. Clumping was noted by Wintrobe[7] in cases of multiple myeloma, kala azar, cirrhosis of the liver, pneumonia and nephritis, and by Gradwohl[4] in three cases of Hodgkin's disease. Kracke and Hoffman[5] reporting a case stated, "that the erythrocyte count was impossible because of autohemagglutination," which was present to a small extent even when normal saline was used as a diluent. The formation of a coarse precipitate in the blood plasma was indicated by Ch'u and Forkner[1] as the cause for erythrocyte agglutination. Shone and Passmore[6] observed that autohemagglutination was found almost constantly in atypical pneumonia being more pronounced in the severe cases.

Case #1. E. G., an army major recently returned from India was seen in our outpatient clinic. Examination of the patient's blood for a routine red blood count revealed agglutination of erythrocytes in Hayem's solution both macroscopically and microscopically. Fresh Hayem's solution which was stored at room temperature (about 22° C.), was placed in a test tube and heated gently over the flame of a microburner by passing the tube slowly through the flame four or five times. When oxalated whole blood was mixed with this warm Hayem's solution in the red counting pipette, agglutination still occurred. Additional heating of the Hayem's solution resulted in no agglutination when mixed with the patient's blood. No record was made of the temperatures of the Hayem's solution but it was not high enough to lyse the erythrocytes. Wintrobe[7] states hemolysis occurs in temperatures above 64° C. The erythrocyte count finally was determined to be 4,400,000 per cmm. and the notation "marked agglutination of erythrocytes" was added to the report.

An examination for cold hemagglutinins was made on a new fresh specimen of defibrinated blood. Cold agglutinins were present in dilutions up to 1 : 1024. A diagnosis of atypical bronchopneumonia, probably of viral etiology was confirmed by X-ray.

Eight days later another specimen of blood showed no agglutination in Hayem's solution, the erythrocyte count being 4,460,000 per cmm. No further cold agglutinin determinations were undertaken.

Case # 2. J. K., was admitted to the sanatorium with symptoms suggestive of tuberculous meningitis having been treated prior to admission with streptomycin.

In attempting to perform a red blood count marked macroscopic agglutination of the erythrocytes in Hayem's solution was observed. The use of physiological saline as a diluent produced no hemagglutination.

In view of the previous experience with the phenomenon of autoagglutination of erythrocytes in Hayem's solution in the previous case, a more detailed record was accumulated. Hayem's solution was heated to varying degrees of temperature and separate samples of the patient's oxalated whole blood were mixed with the various heated portions of Hayem's solution in red blood count pipettes. Agglutination was present in all samples of Hayem's solution heated to temperatures up to 65°C. At 68°C. there was no agglutination nor hemolysis and the erythrocyte count was determined as 3,260,000 per cmm. No visible hemolysis was noted even at 78°C. and the erythrocyte count was calculated to be 3,700,000 per cmm. On the same specimen of blood the erythrocyte count was 3,400,000 per cmm. using physiological saline at room temperature as a diluent. All three counts were within the limit of technical error for the procedure. The hematocrit reading by the Wintrobe method[7] was 38 and the plasma a clear straw color.

A cold agglutinin titration, which was performed on a separate specimen of defibrinated blood to attempt to correlate autoagglutination in Hayem's solution with an elevation in cold agglutinins, revealed none to be present by the method employed.

At autopsy the cause of death was reported as a brain abscess caused by a fungus.

Agglutination of erythrocytes in Hayem's solution occurred in a patient with a typical bronchopneumonia probably viral in origin, and in one with a mycotic brain abscess. The hemagglutination was prevented by raising the temperature of the Hayem's solution. The accuracy of erythrocyte counts after such a procedure was found to be substantially reliable since subsequent red blood counts, where there was no agglutination in the diluent, were almost identical.

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SOME LEGAL ASPECTS OF MEDICAL TECHNOLOGY*

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As knowledge increases, the fraction of the entire amount of it that each person can know grows smaller. Fortunately it is not necessary that any one person be master of all the knowledge of the race, to make all that knowledge available for the good of each individual member of the human family. What is required is a wide division of labor, both physical and mental. The whole tree of knowledge grows not so much by each limb's or branches' increasing in size, as by an increase in the number of limbs and branches.

Thus in healing, curing and relieving, or in attempting to heal, cure and relieve, those suffering from injury or deformity or disease of mind or body, no one person combines in himself all the skills and sciences a modern world applies to the accomplishment of that purpose. We have the general practitioners of medicine and also the specialists, and in addition to those who work entirely within the field of medicine, whole armies working in the fields of natural sciences, physics, chemistry, botany and zoology, each with its many, many branches—all of them fitting the results of their study and experience into a tremendous organization, through which the whole body of human knowledge is laid under the obligation to supply its service to the most obscure sick or wounded man, woman or child.

*Read before ASMT Convention, June, 1953, Louisville, Ky.

Through this whole structure runs like quick-silver, certain principles or rules of conduct of each person in his relationships with all other persons. These principles and rules in the aggregate are known as the law. It too, has many divisions. Only one of them will be considered in this part of the program of this Convention. That will be the law in medical technology.

One of the general rules of law regarding the conduct of persons in their relationships and contacts will all other persons is that each should use the amount of care and caution used by other persons of ordinary prudence and ability under the same or like circumstances, to avoid injuring other people. That is a general statement of the law of negligence. If one fails to use that degree of care and another suffers injury as a result thereof, the law will exact from the careless one, compensation for the injured one. This general rule of law is modified to fit specific cases where any obligation to use special skill and ability is involved. The adaptation of that rule to those caring for the sick corresponds with the particular type of skill and training required for proper care.

To illustrate: If one holds himself out as a general physician and undertakes the general practice of medicine, then the person who employs him as such has the right to rely upon him to have and use the skill and care ordinarily used by general physicians practicing in the same or similar localities. If one holds himself out as a specialist, then the one employing him has the right to expect him to have and use the skill and ability ordinarily used by specialists in the same field. So if a technologist is employed in the team working together in the interest of the health of a person, that person has the right to expect the technologist to have and use the skill and care that is ordinarily used by technologists rendering the same kind of service. The same is true of nurses and ot anyone else connected with the protection or restoration of one's health. All are measured by that same standard—that is, the standard of the usual care and skill of those engaged in rendering the particular service in question. That is true whether the service is of a professional or a non-professional kind.

In the field of health service the lines drawn between professional services are not always so sharp and distinct that what falls within one profession and not within another can be determined with certainty. The general guide for the determination of the boundary lines of each profession is to be found in what each profession is trained to do and ordinarily does. So these lines are constantly changing. For instance, at one time the administration of a hypodermic was regarded as a surgical procedure. Now it is regarded as a nursing procedure. Drawing blood, until recently, was regarded as a medical act. Now it may

or may not be. Yet there still remains a large field in medicine in which the law clearly requires the practitioner to have a license to practice.

Without spending more time in abstract or general statements of the law in relation to medical technology, specific problems in that field will now be given some consideration.

1. Regarding Blood Bank Problems

The technologist generally is an important professional workman in the operation of blood banks. Care is required in every step of that work. The drawing of the blood must be done with proper precaution against infection of the donor, against unnecessary pain, and against any other unfortunate consequennce to the donor that could attend lack of skill and knowledge. The blood itself must be handled in accordance with the standards generally complied with in that regard. The labeling for identification must be done with a high degree of accuracy and consideration for all the results that could follow from carelessness, and carelessness must be avoided.

The typing and classifying of the blood with respect to every point involved must likewise be done by one who has all his wits about him in doing that work, and mistakes must be avoided—for they might be fatal. Cross-matching falls into the same class of acts to be performed with great care.

However, even in these highly important matters the same general rule governs as to what is negligence that has already been stated. That is, negligence is the failure to use the care and skill that would ordinarily be used by a technologist in doing these things. The average level of such care and skill is very high. Therefore, to come up to the average, great care and skill must be used.

What has been said regarding the work of the technologist applies with equal force to the work of the clerks, record keepers, nurses, technicians, and physicians, who use the blood in performing their services or who keep records and accounts or who transport and deliver the blood to be used. This duty to use great care and skill cannot be too forcibly impressed upon the people who do such work.

2. Promptness in Making Reports

Negligence in the performance of the duties of a technologist may consist in delay in making reports of tests or other investigations. There are situations which require immediate tests and reports for the safeguarding of the life of a patient. Where that need is, or should be, obvious to the technologist, or where the technologist is informed of it by the physician or anyone else, the technician should act on that information. The time within which tests or investigations may be made and reports given

in order to avoid a charge of negligence, is established by the same general test of negligence already discussed. That is, it should all be done within the time that would be required by technologists using the skill and care generally used in the same or like circumstances.

The simplicity of these statements should not mislead anyone as to the difficulties that may attend the application of them to the work to be done. Suppose, for instance, the technologist is already busy with the kind of work that requires prompt completion for the welfare of a patient; and that at that time other work of an urgent nature is submitted. What should the technologist do? Weigh the comparative urgency of the two jobs to be done and do the one that is found to be more urgent? Or do the work in the order in which it came? The only guidance that can be given him is that he should do what the ordinarily cautious and prudent technologist possessing ordinary skill and ability would do under the same or like circumstances. Whichever choice he makes might result in a law suit, if either patient suffers or dies before a delayed report is given, if an earlier report would have avoided the unfortunate result.

And if there is a law suit, who will decide what he should have done? The jury, if a jury tried the case—the judge, if the case was tried without a jury. Their decision should be upon the evidence in the case, and if there is conflicting evidence their decision should be in accordance with the preponderance, or greater weight of the evidence. Fortunately evidence in a case of this kind would be confined to the testimony of those who were engaged and had experience in the same profession. The standard of care in regard to relayed reports, just as in regard to any other question that might arise within the practice of medical technology, could be established only by expert witnesses. To qualify as experts the witnesses would have to be technologists, in a case where the standard of technological service would be an issue in the case.

What then can a technologist do to protect himself against liability on account of not getting his work done because he had too much to do? He should inform those demanding his services regarding the time when he can do the work. If he cannot get it done in time to meet an emergency that might be developing, he should inform the physician, or whoever else may be demanding the services, of that fact so there may be opportunity to obtain additional help, or so that the physician, if he is able to do it, may do the work himself. He should do what the ordinarily cautious and prudent technologist having the usual skill and ability of members of that profession would do under the circumstances.

The technologist's obligations run to the patient rather than

to the doctor when the question of liability for injury to the patient is involved. The patient may maintain an action against the technologist for his failure to conform to the proper standard even though the physician may have suggested to him that she follow the course that would be a departure from the proper standard of technological service. In a situation of that kind both the physician and the technologist could be held liable on the ground that each of them failed to conform to the proper standard of service in the profession in which each was engaged.

Of course, one may protect himself against liability by insurance against malpractice, which means negligence in the performance of professional duties.

3. Accuracy in Reports

The technologist's reports should not only be sufiiciently prompt to meet the requirements of the situation, but they should be accurate, at least to the degree that the usual standard of such service requires.

Reports may be made as comprehensive as the training and judgment of the technologist would make possible. While the technologist does not have the responsibility to make a medical decision, yet if the report includes medical decisions or conclusions, that alone will not get the technologist into difficulty on the ground that he is going outside of his field and is practicing medicine. Of course if he made medical decisions and prescribed upon the basis of those decisions and charged the patient for it, and ceased to act only as one obtaining the data upon which the physician should carry on the treatment and bear the responsibility as the physician of the patient, then he would be engaged in the practice of medicine, and if he had no license to practice medicine his conduct would be unlawful. As long as he confines himself to the obtaining of the data and the reporting thereon, and advising and counseling the physicion, even to the extent of giving his opinion to the physician as to diagnosis and treatment and his medical interpretation of the data on which he has found and reported, but does not perform the medical acts of prescribing medicine or regimens for the patient, he cannot be in difficulty with the law on the ground that he is practicing medicine.

Of course he may be in difficulty with the physician if he assumes to give medical counsel and advice when the latter does not seek it. A guide to his professional relationships with the physicians can be found in a statement of Confucius. He said, "He who gives advice where it is not sought, himself standeth in need of counsel."

But this part of the program of this Convention is being devoted to the legal aspects of medical technology, and not to

inter-professional relationships of those who are on the team to supply the care of the patient.

Keeping in mind both the fact that the interest of the patient should be the first consideration of all who participate in any activity that may affect his welfare, and also the fact that there are boundary lines between the professions of nursing, medical technology, the practice of medicine, and other professional activities within this field—the medical technologist should at least hold himself strictly accountable for care in obtaining the data and in the accurate reporting of it. The law will hold him accountable for service that fails to comply with the professional standards in this part of his work just as in all other parts of it.

What has been said should not be construed to mean that the technologist may wander all over the entire field of good advice, counsel, conclusions, and medical decisions, and make a technological report that would consist only of such matters. What is meant is that even if those matters are included in the report it will not necessarily make the report inadequate. But the essential elements in a report to make the report adequate should be the statement of the facts or data obtained, in the form in which those for whom the report is prepared can use it in reaching their own conclusions. To illustrate: The report should show what was found by the microscopic study, what was found in the blood count, what was found in the biological test, and so on through the whole range of the technological service. What the data found and reported would support in the way of an inference or conclusion regarding the diagnosis of a condition, is not within the field of medical technology. That is within the field of medicine.

4. To Whom the Report Should be Made

This sub-topic is of less consequence from the legal aspect than from the practical business aspect. Statutes generally do not require physicians to keep confidential the information they gain regarding individuals in the practice of their profession. The statutes upon that subject are generally stated negatively. They provide that physicions and certain others occupying confidential relationships are not competent to testify in a court. The word "competent" in that sense means that they cannot be compelled to testify because the statute makes their testimony regarding these confidential matters inadmissible in evidence.

The obligation of physicians to keep professional information confidential is one of the elements in the physician-patient relationship that is implied from custom and usage. The tradition of the physician keeping confidential information regarding his patients was already well established when Hippocrates included in his "Hippocratic Collection" the famous passage known as

the Hippocratic Oath, 400 years B. C. This sentence is part of that oath,

> "Whatsoever things I say or hear concerning the life of men, in my attendance on the sick or even apart therefrom, which ought not to be noised abroad, I will keep silence thereon, counting such things to be as sacred secrets."

That the technologist should not noise abroad the information he obtains in the practice of his profession concerning his patients will be readily accepted as sound ethics. But when it comes to whether he should make a report directly to the patient or make the report only to the physician and refuse to make it to the patient, presents a more difficult problem. Here again the requirements of practicality and good business throws some light on the solution of the ethical or moral problem. The technologist performs the service for the benefit of the patient, but with the idea in mind that the information will be used by the physician as the means to determine what adequate care and treatment should be. He is not generally hired by a layman to supply data on which the layman may make decisions in regard to his own treatment. Any technologist should confine his services within the usual scope and boundary of the technologist's service.

The making of the diagnoses and the informing of the patient of what the diagnosis is falls within the sphere of the physician's services. So the conclusion is that the report should be made to the physician and not to the patient—unless the employment of the technologist comes directly from the patient with the patient stating, and contracting with the technologist for, the service the technologist is to render. Where the employment has been made by the physician the technologist reports should be to the physician, but where the employment has been made by the patient then of course the technologist deals with and reports directly to the patient.

This latter type of activity, however, is one which the technologist should engage in only with careful precaution against making reports anything more than merely reports of factual data without medical conclusions and inferences. If the reports go so far as to constitute diagnoses or prescriptions, then the technologist is practicing medicine, and if not licensed to do so would be in violation of the law.

From all this it is obvious that the safe practice for the technologist to follow is to accept employment only from physicians and then make the report only to the physicians.

5. Who Are Liable for Mistakes in Technological Service?

Where injury results from negligence of a technologist of

course the technologist is liable. But there may be mistakes in technology where others may likewise be liable. A few instances will be given.

(1) The master is liable for the negligence of his servant; the principal, for the negligence of his agent; and a partner, for the negligence of another partner—as a general rule. If the technologist is employed in a hospital that is operated for profit, the corporation, partnership or individuals who own and operate that hospital would be liable for the negligence of the technologist. But if the hospital is not operated for profit but is a charitable institution, then the owners and operators of the hospital are not liable for the negligence of the technologist if they had used ordinary care in employing the technologist. In that case the employer would be liable only for a failure to use proper care in the employment of the technologist as a servant to perform services given in the hospital.

(2) If the physician or surgeon in general charge of a patient fails to give proper instructions and supervision where necessary regarding the work of the technologist, and the technologist as a result of that failure makes a mistake, then if the mistake was of the kind that the ordinarily cautious and capable technologist would not have made, the technologist is liable for his own careless and negligent conduct, and the physician or surgeon is likewise liable for the failure to give the supervision and instructions necessary, if the ordinarily cautious and prudent physician under the same or like circumstances would have given instructions and supervision.

(3) If the technologist has a student assistant who makes a mistake because the technologist failed to give instructions and supervision of the kind that would be given to an assistant by a technologist of ordinary skill and ability under the same circumstances, then the technologist is liable for the mistake of his assistant. And the assistant might also be liable if the mistake was one that would not have been made by the ordinarily cautious and prudent assistant under the same or like circumstances. Of course if the technologist has properly instructed a student assistant and the student assistant then fails to follow the instructions and injury results, the student assistant and not the technologist would be liable.

The liability that one may incur for negligence may arise from acts of omission as well as from acts of commission. The failure to do a thing that should have been done, and the doing of a thing that should not have been done, are of equal significance in the law of negligence.

Where the technologist steps out of his own field and enters into the field of the practice of medicine he is held to the same standard of care as if he were a physician. In other words, if one

undertakes to render medical service, even though he has no license, he has the duty to render service that conforms to the same standard of skill and care as is required if he has a license. This is true even though practicing is the violation of a criminal statute. If one undertakes to do a thing that requires special skill and ability he cannot be excused from liability for poor service merely because he does not possess that skill and ability.

Conclusion

Medical technology is a very new profession. It is bound to increase in importance. As new techniques are developed new skill and understanding in those techniques will follow. The division of labor that has produced in medicine and general practitioner and the wide range of specialists it seems will also develop in medical technology; and the time, if it is not already here, may soon arrive when there will be general medical technologists and also specialist medical technologists.

Professor Breasted, head of the Department of Oriental Studies of Chicago University, says in his History of Ancient Peoples that about the middle of the 19th Century, that would be about 1850, marks the beginning of the tremendous expanse of knowledge which has resulted in greater advancement in medicine and all other fields of scientific activity than had taken place in the whole preceding period of recorded human history. He made that statement about 1916. At that time no atomic energy had been released. No radio had carried the sound of a human voice. No radar had ever directed a search light nor sighted a gun. No electronic device had ever opened a door. Sulfas, penicillin and other miracle drugs were unknown. Whole industries based upon new discoveries and increasing scientific knowledge have developed since 1916; and so much has taken place since that time that even that date seems an ancient one when consideration is given to all the things that have transpired since 1916.

These recent achievements, and the vast numbers of men and women who are directing their intellectual energies into scientific pursuits, and the eagerness with which business makes the results of their researches available to all mankind—prove that this expansion, whose point of beginning Professor Breasted fixed at about 1850, has lost none of its original force, but is gathering additional power and momentum. As medical technologists, you are to be congratulated on being in that brilliant army of the scientific minded who are making science useful in the fight against disease and the preservation of human life.

Of those who fight to save life rather than to destroy it, a great doctor, Oliver Wendell Holmes, had this to say:

> "The valiant stand outside and wait
> Till all their scars are shown—
> These walk unchallenged through the gate
> And sit beside the throne."

THE LABORATORY CONTROL OF ANTICOAGULANT THERAPY: FACTORS DETERMINING THE ACCURACY OF ONE-STAGE PROTHROMBIN TIME DETERMINATIONS*

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As recently as ten years ago, the various questions concerning the physiology and biochemistry of blood coagulation were largely associated with the research laboratory. A rather simple and straight-forward scheme was sufficient to explain the coagulation of the blood under normal conditions, and the presence of certain circulating anticoagulants, such as heparin, sufficed to explain the fluid state of the blood while in the body. The classic and rather simple explanation of blood coagulation as a two step process involving the interaction of thromboplastin with prothrombin in the presence of ionic calcium to form thrombin, and the subsequent reaction of this thrombin with fibrinogen to form fibrin answered most of the questions. It had been established that the liver was the site or origin of certain of the chemical substances which entered into the coagulation reaction, and it was known that patients suffering from certain types of liver disease showed abnormalities in the clotting phenomena.

About fifteen years ago the first work was being reported upon the action, and eventual isolation, of a new vitamin, Vitamin K, which was found to be essential in the production of prothrombin by the liver. The use of Vitamin K as a therapeutic agent in certain blood disorders, and as a prophylactic agent to prevent certain diseases, particularly certain hemorrhagic diseases of the new-born, began to increase. At this time, two methods for the quantitative determination of prothrombin in the blood had been developed. The one-stage method of Quick,[1] and the two-stage method of Warner, Brinkhous, and Smith.[2] Neither test was commonly performed in the clinical laboratory, and the results obtained were rather erratic due to the lack of standardization of the reagents, and of techniques. The one biggest variable was probably the thromboplastin. Different laboratories were not able to obtain thromboplastins of equal activity following the complete directions for acetone extraction of rabbit brain, and when we consider that in addition to rabbit brain thromboplastin, material was also being obtained from rabbit lung, from lung and brain tissue of other species, and from completely non-mammalian sources such as reptile venom, it is not

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surprising that the results obtained from so-called prothrombin time tests fifteen years ago were somewhat erratic, and it was almost impossible for any two laboratories to compare results.

With the advent of Dicumarol as an anticoagulant agent it became evident that rigid laboratory control of this treatment was necessary. As it was determined that Dicumarol affected the liver in such a way as to prevent, or retard, the formation of prothrombin, the clinical laboratory was suddenly faced with the necessity of doing many prothrombin time determinations with sufficient accuracy to avoid hemorrhagic complications due to Dicumarol overdosage. Whereas fifteen years ago prothrombin time determinations were a research laboratory curiosity, they have now become routine procedure for every clinical laboratory. Unfortunately, however, the various factors which tend to give variable results in this prothrombin time test are still present, and strict standardization of reagents and technique is necessary if reproducible and reliable results which can be compared with those of other laboratories are to be obtained. No attempt will be made here to cover all of the various factors which play a role in coagulation phenonoma. Various accelerator substances and an almost equal number of inhibitory substances have been suggested. Only those which are of primary interest to our present discussion will be mentioned.

In 1946 and 1947, various investigators reported that a heretofore unrecognized blood constituent was necessary for the normal and rapid coagulation of the blood. The conversion of purified prothrombin into thrombin by purified thromboplastin in the presence of calcium was shown to be very slow and a new factor, called "labile factor" by Quick,[3] "factor five" by Owren,[4] and "accelerator globulin" by Seegers and his co-workers,[5] was postulated. As is evident from the name given this factor by Quick, the substance is labile and disappears from stored plasma rather rapidly. It was found that the increase in prothrombin time which had been reported for stored plasma, and which had been interpreted as a disappearance or decrease in activity of the prothrombin was largely a disappearance of this labile factor. The addition of active accelerator globulin to stored plasma will restore the prothrombin time to normal, or nearly so. This picture has been further complicated by the recognition that accelerator globulin may be present in two forms. As it circulates in the plasma it is relatively inactive and is converted by very small amounts of thrombin to a more active form which has been called serum accelerator globulin.[6] Serum accelerator globulin is probably identical with Owren's factor six. Serum accelerator globulin is very active in catalyzing the reaction of prothrombin with thromboplastin to produce thrombin.

The last stage of the coagulation process is the classic one of the change of fibrinogen to fibrin under the influence of thrombin. Which of the various reactions indicated here are enzymatic, and which compounds are consumed during the various steps of the coagulation process is still unsettled.

The simple, two-step scheme for blood coagulation has been replaced with a somewhat more complex mechanism which is shown here.

$$\text{Prothrombin} + \text{Thromboplastin} \xrightarrow{Ca^{++}} \text{Thrombin (Slow)}$$

$$\text{Plasma Ac-globulin} \xrightarrow{\text{Thrombin}} \text{Serum Ac-globulin}$$

$$\text{Prothrombin} + \text{Thromboplastin} \xrightarrow[\text{Serum Ac-globulin}]{Ca^{++}} \text{Thrombin (Rapid)}$$

$$\text{Fibrinogen} \xrightarrow{\text{Thrombin}} \text{Fibrin}$$

This scheme may still be too simple since other factors modifying the coagulation mechanism have been suggested, such as the "Serum Prothrombin Conversion Accelerator" of Alexander,[7] and others too numerous to mention here. The practical importance of these new factors was well outlined by Alexander.[8]

There are two classes of anticoagulants used clinically—the one represented by heparin, the second by Dicumarol. Heparin acts as an anticoagulant in possibly three different ways. It has been suggested that heparin prevents or markedly impedes the agglutination and disintegration of the blood platelets, and thereby prevents the liberation of thromboplastin, interfering with the first step of the blood coagulation scheme. Secondly, it combines with a plasma co-factor to inhibit the conversion of prothrombin to thrombin. Thirdly, it seems to form with a serum albumin, a strong antithrombin, which interferes with the last stage of the coagulation process, the conversion of fibrinogen to fibrin. The administration of heparin to experimental animals and to human beings by intravenous administration has a direct and immediate effect upon the coagulability of the blood as reflected in the prolongation of the Lee-White coagulation time. On theoretical grounds heparin should be an ideal anticoagulant for use in the prevention of thrombotic phenomena inasmuch as it is a naturally occurring substance and comparatively nontoxic. It has, however certain disadvantages. First, it must be injected either intravenously, or intramuscularly in the form of a slowly absorbed complex, and secondly it is rather rapidly destroyed or inactivated in the body so that injections must be made frequently or administration must be by intravenous drip.

These difficulties incident to the use of heparin as a therapeutic agent have prevented more wide-spread use. The action of heparin may be controlled by determining the coagulation time of the blood by the Lee-White method, or by that of Weiner and Shapiro.[9]

The second type of therapeutically active anticoagulants, the first of which was dicumarol, has no action in vitro. The addition of dicumarol to shed blood will not alter the congulation time, and the coagulation time of the blood of a patient receiving dicumarol is essentially normal. As was mentioned previously, this group of anticoagulants reacts by interfering with the production of prothrombin by the liver. There are four compounds with roughly similar physiological action in clinical use at the present time. All four compounds act as anticoagulants upon oral administration, and as far as we know at the present time, all act upon the same fundamental enzyme system in the liver in such a way as to prevent or retard the production of prothrombin. Therapy with all four compounds, Dicumarol,® Danilone,® Tromexan,® Cumopyran,® is controlled by prothrombin time determinations.

Let us return then to the primary subject of this discussion, namely, the laboratory control of anticoagulant therapy.

As mentioned, the usual laboratory control of therapy with dicumarol and the other similar chemical compounds is the so-called one-stage prothrombin time test. The basic technique of this test may be summarized as follows:

Nine volumes of freshly drawn blood are added to one volume of sodium oxalate. The blood is then centrifuged, the plasma removed, 0.1 cc of this plasma is added to 0.1 cc of thromboplastin and 0.1 cc of calcium chloride, and the time of clotting determined. The above method would seem to be extremely straight forward and simple, and it is astonishing how many errors can creep in and how many different results can be obtained by using what would seem to be identical techniques. Most of these variables can be eliminated, or at least minimized, by proper laboratory technique.

The first problem is inherent in the test itself—the "so-called prothrombin time determination." In most tests which the laboratory is asked to carry out, one can be reasonably sure that the test is measuring the particular factor in which the clinician is interested. In performing the one-stage prothrombin time determination exactly the opposite is true. We are sure that the one-stage prothrombin test is not measuring the level or the acivity of the prothrombin in the blood. We are, rather, measuring "total potential coagulability" of the blood, and this is a summation of the activities of the prothrombin, the accelerator glob-

ulin and the other potentiating factors balanced by activity of the various inhibitory factors which may interfere with the reaction. For the above reasons, the modified two-stage technique, as presently used by Seegers and his associates,[10] is a more reliable measure of actual prothrombin activity because an attempt is made to control all of the other factors, including accelerator globulin. For practical use, however, in the clinical laboratory the two-stage procedure is rather time consuming. Fortunately, for both the laboratory and the clinician, the more specific results obtained from the two-stage test are not necessary for the control of anticoagulant therapy. The clinician wants to know if the amount of anticoagulant being given to this particular patient is adequate to maintain the total coagulability of the blood at such a level that neither spontaneous thrombosis nor hemorrhage will occur. Fortunately, the various modifications of the one-stage prothrombin determination, in spite of any theoretical drawbacks, seem completely adequate to answer the above questions—providing the test is being done properly and with the same technique day after day.

A good example of the anomalous results which may be obtained with the one-stage prothrombin time determinations was recently given by Brambel.[11] They were asked to study the blood from a leukemic patient. By the one-stage method, using undiluted plasma, they obtained a prothrombin time of 40 seconds. Carrying on their investigation, they took another blood sample from a patient receiving dicumarol which also gave a prothrombin time of 40 seconds. The obvious conclusion would be, therefore, that the prothrombin activity of these two patients, one suffering from leukemia and one being treated with dicumarol, were identical. Were they? When the two plasmas were diluted with saline to get a 50% dilution, the leukemic plasma gave 64 seconds, and the dicumarolized plasma 67 seconds. Under these conditions, the two plasmas were, to all intents and purposes, still identical. As a further check, each of these plasmas was diluted with an equal volume of prothrombin-free normal plasma having a prothrombin time of 14 to 15 seconds, and the one-stage prothrombin test repeated on the two mixtures. In the case of the leukemic patient, the prothrombin time was now 26 seconds whereas in the case of the dicumarolized patient the prothrombin time was 66 seconds. Under these experimental conditions, the two samples are obviously not identical in activity. Using the original two-stage procedure on the two plasmas, the leukemic plasma gave 53% activity, and the plasma from the dicumarolized patient 39%. This difference is not particularly striking as it represents 153 thrombin units per cc as compared to 103 thrombin units per cc. If, however, acceler-

ıtor globulin is added to the two-stage procedure the following results were obtained: Leukemic plasma 85% or 205 thrombin units per cc, and the dicumarol plasma 46% or 128 thrombin units per cc. From this series of experiments, then, it is obvious that the one-stage method on the whole plasma was not measuring the same thing in the two different samples. Using the modified two-stage technique with the addition of adequate accelerator globulin, the leukemic plasma gave essentially normal results for prothrombin activity whereas the dicumarolized plasma was low. It is recognized, however, that from the clinical standpoint both of these patients exhibited potential bleeding tendencies. While the so-called prothrombin time test was not specifically measuring prothrombin, it did give a relatively true picture of the potential coagulating ability of the two plasmas. The first thing to recognize then is that the test which we call the prothrombin time test is not highly specific and is really misnamed.

In spite of the above academic discussion, the one-stage prothrombin time test will be used for a long time in the clinical laboratory, and it will be worth while to investigate some of those variables which are subject to control.

The several points which will be covered are those in which variations in technique or standardization of conditions in performing the test have been shown to affect the results of the one-stage prothrombin time determination. These various factors represent the accumulation of data obtained in our own laboratory and from various clinical laboratories with whom we have been working. In most laboratories the same one or two technicians always do the prothrombin determinations. As one pathologist put it, "If the same technician does the test today, I am reasonably sure that she will make the same errors as she made yesterday and will make tomorrow, and the results will be comparable." This is rather a backhanded way of solving the problem, but unfortunately it is close to the truth in many cases.

The first source of error can creep in at the very start in the taking of the blood sample. If the venipuncture is not clean, and if several stabs have to be made before a good flow of blood can be obtained, it is unavoidable that contamination of the blood sample with tissue juices will occur. Tissue juices containing variable amounts of thromboplastin and other potentiating substances which may have considerable effect upon the ultimate results of the prothrombin time test, particularly if the plasma stands very long before the test is performed. There may be a partial conversion of prothrombin to thrombin due to the tissue contamination, and this will reflect in the results. This

can be a major source of error.

Another error which occasionally occurs is variation in the amount of oxalate added to the blood. As little as a 10% variation in oxalate concentration can make a substantial difference in the ultimate prothrombin time. The method calls for the addition of nine volumes of fresh whole blood to one volume of 0.1 molar sodium oxalate. For example, proper oxalation gave a prothrombin time of 15 seconds. If less oxalate is used, as might result from adding too much blood to the centrifuge tube, the prothrombin time may be slower, or faster, depending upon the length of time the plasma stands before the test is run. If 10% too much oxalate was used, that is, too little blood, the prothrombin time was 18 seconds, and if a 20% surplus of oxalate happened to be present, the time rose to 19 seconds. Too much oxalate is the more common error. In some instances the vein may collapse before the requisite amount of blood is drawn, and if the amount of blood already taken is pretty close to the required amount it may be used and, therefore, result in increased oxalate concentration. In spite of the hope that such things do not happen, the fact is that they occasionally do. The correct oxalate-blood ratio is important because some of the calcium which is added later, in the determination of the prothrombin time, is used up in neutralizing the excess oxalate. Variation in oxalation, therefore, is equivalent to varying the strength of the calcium chloride solution which is being used in the actual performance of the test.

The next factor is the effect of centrifugation upon prothrombin time. All the various tests associated with blood coagulation studies emphasize the desirability of minimum centrifugation, and 5 minutes at about 2000 rpm seems to be a good figure. Ten minutes centrifugation may increase the ultimate prothrombin time slightly, representing an apparent loss of prothrombin activity. Longer centrifugation gives gradually increasing prothrombin times until it seems to plateau at about 20 seconds. Short centrifugation at higher speeds will have a similar effect. Therefore, it can be stated that centrifuging the blood for a short time at a comparatively low speed is essential if reliable and reproducible prothrombin time results are to be obtained. Too little centrifuging, however, will also yield erratic times and falsely high prothrombin activities (fast times) due to a varying number of platelets remaining in the plasma.

It has been repeatedly stressed that the plasma should be separated from the cells immediately following centrifugation, and the prothrombin time tests should be performed as quickly as possible. Under actual working conditions, there is little loss of prothrombin activity in the first hour or two if the plasma

stands at room temperature. It has, however, been observed that dicumarol plasma is less stable than normal plasma, and shows a greater tendency to lose activity with standing. It is usually recommended that if the tests cannot be performed within two hours after the blood is withdrawn that the plasma be placed in the refrigerator until used. Higher temperatures, however, such as that of a water bath at 37° C, will alter the prothrombin activity rather rapidly. This is not a particularly common source of error under normal conditions. It has, however, occurred in some instances where a series of prothrombin time tests were started, the tubes of plasma put in the water bath to reach 37°, and then through some unavoidable circumstance the technologist was called away and left the tubes in the bath. The plasma should remain in the water bath no longer than necessary to bring it up to 37°, and several plasmas should not be placed in the water bath at one time to stand, unless one is sure that the tests can be completed on all samples within a comparatively short space of time. One hour at 37° will destroy 50% of the prothrombin activity of normal plasma, and an even greater percentage of the activity of dicumarolized plasma. This phenomena has shown up several times in different laboratories where comparative work was being done on the activity of several different types of thromboplastins. A series of prothrombin time determinations were performed on several samples of plasma with thromboplastin A, and after this was finished the same samples were run with thromboplastin B. The plasma, however, had stood in the water bath during this time, and the results obtained with the second thromboplastin did not check with those obtained with the first, the times being considerably longer. It was found that about one-half hour had elapsed between the first prothrombin determination with thromboplastin A and the comparable determination with thromboplastin B, and a considerable loss of activity had occurred.

Another source of error, and one which occurs occasionally, is the effect of variations in water bath temperature upon the test itself. A variation of 2 to 3 degrees may result in a 5 to 10 per cent difference in prothrombin activity—slower times at lower temperatures, and vice versa. Actually, as far as the reproducibility of the results of any one laboratory are concerned, it makes no difference what temperature is used as long as it is always the same. However, the results obtained under these conditions cannot be properly compared with the results from some other laboratory in which the temperature has been maintained at 37°. In many laboratories the tube in which the reaction is taking place is removed from the water bath to observe the end point, and from all practical standpoints

if the reagents, plasma, calcium chloride, and thromboplastin, were at 37° when mixed, the actual process of coagulation can be observed outside of the water bath. The short time interval involved will not alter the temperature significantly.

Another source of variation is the effect of different concentrations of calcium chloride. Altering the concentration of calcium chloride will alter the prothrombin time of normal plasma, and may change the shape of the curve slightly. Here again, the important point is the realization that the calcium chloride solutions should be prepared accurately and that the same strength of solution should always be used if the reproducible results are to be obtained. Two commercially available thromboplastic materials, Schieffelin's Solu-Plastin®, and Warner-Chilcott's Simplastin®, adequately control this factor by supplying accurately prepared calcium chloride solution in the case of Solu-Plastin®, and the incorporation of the calcium chloride into the thromboplastin in the case of Simplastin®. The proper concentrations of calcium chloride for optimum results in prothrombin determinations has been a moot question for some time. It has been shown by Quick,[12] as well as by others, that the optimum concentration for normal whole plasma is not the same as the optimum concentration for dicumarolized plasma. The lower the prothrombin activity of a given plasma, the greater the amount of calcium needed for optimum clotting. Obviously, one cannot keep changing the calcium chloride concentrations to fit the various plasmas being tested so that the data must be referred to some one calcium concentration. Results under these conditions should be reproducible.

Another source of variation may be found in the setting up and determination of a plasma dilution curve to which experimental results will later be referred. Figure 1 shows two plasma dilution curves determined on 25 normal plasma samples using saline as the diluent in one case, and barium sulfate plasma, that is, prothrombin free plasma, in the other case. As can be seen, as the per cent of prothrombin decreases there is an increasing divergence in the curves, and also an increasing deviation in the results obtained. This difference in the two curves is probably a result of several factors. The first is unquestionably the effect of diluting the other blood coagulation constituents with the saline so that there is an appreciable dilution of accelerator globulin, fibrinogen, and a shift in the general physical chemical make-up of the reaction mixture. The other factor is the increasing difficulty in determining the end point, since with the decrease in fibrinogen in saline diluted plasma the end point becomes progressively more difficult to determine as a firm clot does not form. This last factor probably explains the in-

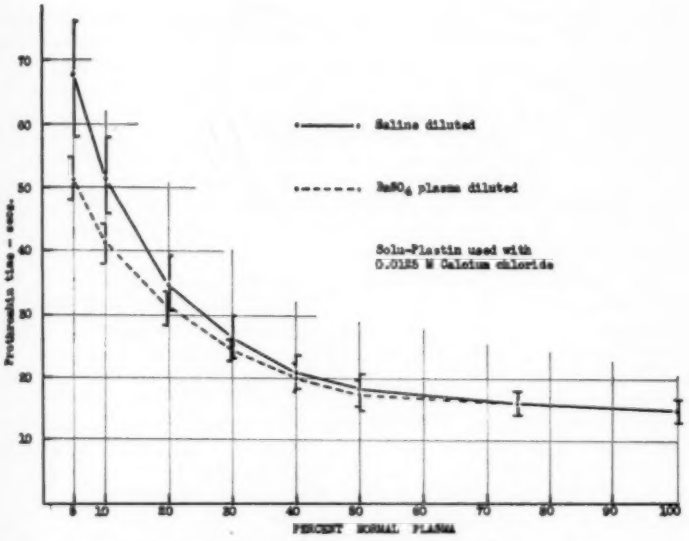

Figure 1. Effect upon one-stage prothrombin times of normal plasmas diluted with saline and prothrombin free ($BaSO_4$) plasma

creased variation in results in the five, ten, and twenty per cent plasmas. For optimum results with greatest accuracy and reproducibility, it is now generally believed that prothrombin free plasma is the best diluent for the setting up of the plasma dilution curve. Barium sulfate plasma or prothrombin free plasma is prepared by absorbing normal oxalated plasma with 100 mgs of barium sulfate per cc of plasma for ten (10) minutes at 37°. The barium sulfate is then removed by centrifugation.

This brings up the question of how to read the end point, and here again we find a situation where any method is correct as long as it is always done the same way. The tube may be tipped or rotated or tapped with a finger, or a nichrome wire loop may be used. The end point may be taken as the appearance of definite fibrin particles or the presence of a definite solid clot. In our laboratory, the nichrome loop is preferred as our end points seem to be sharper and we get better reproducibility, particularly where more than one individual performs the test.

A recent publication by Loesner and his co-workers[13] outlines the use of the photoelectric cholorimeter in the determination of prothrombin time. This method would seem to have considerable merit. While they used a Coleman Jr. spectrophotometer it would seem that any similar instrument which could be

adapted for small tubes would produce equally good results. The test is performed in exactly the same way as a normal prothrombin determination except that the tube is placed in the instrument and as soon as the fibrin forms, there is an immediate swing of the galvanometer. The end point is the time in which this swing starts. It avoids all of the difficulties incident to the subjective reading of the end point, and is particularly useful in those plasmas in which a poor clot forms. Any laboratory having this, or a similar instrument, could profitably investigate this method of performing prothrombin time determinations.

The question of how the results should be reported is one of individual preference as far as the laboratory and the clinicians associated with it are concerned. Whether the results are converted to per cent of normal prothrombin activity or given to the clinician in terms of seconds as compared to the normal values, makes little difference. In any case, the clinicians should be familiar with the type of response being obtained by the laboratory, and even though the reports are given in time, the laboratory should have constructed a plasma dilution curve in order to know that they are getting the proper results with their thromboplastin and with their technique.

After looking at a prothrombin dilution curve (see Figure 1), many of which have been published in the literature as well as in the various brochures supplied by pharmaceutical houses, it seems almost incredible that some laboratories are still calculating their prothrombin activities by dividing the normal time by the patient's time, and using that as per cent of prothrombin activity. This, of course, is the method of reporting results from colorometric determinations, but is only applicable where there is a straight line relationship between the concentration and the reading. Completely erroneous values for prothrombin activity are obtained by such arithmetrical manipulation. It is possible to develop a formula, taking into consideration the changing slope of the curve, and express the results mathematically, but it seems much easier to refer the results directly to the curve if results are to be reported in terms of prothrombin activity.

One of the important variables is the thromboplastin being used. Reliable thromboplastins are commercially available: Thromboplastin-Difco, Simplastin®-Warner-Chilcott, and Solu-Plastin®-Schieffelin. Many laboratories still make their own, although it would seem to be a costly procedure and the resulting preparations are never as uniform. The Difco and Warner-Chilcott products are supplied in the form of a dry powder and must be put into solution and their normal activity determined. The necessary calcium chloride is incorporated in the latter

preparation. The Schieffelin product is supplied in solution, with a like volume of calcium chloride solution. The stability of this product is such that many laboratories dispense with daily normal controls, although occasional controls should be run, and certainly on each new package. As a matter of practical consideration, a laboratory running many prothrombin determinations a day will usually have one or two normal plasmas in the series which serve as automatic controls. One point should be made; regardless of the thromboplastin used, whether it is some commercially available product, or a thromboplastin prepared in the laboratory, the same preparation should be used at all times, and if a shift is made the laboratory should realize that absolute time in seconds obtained with various thromboplastic materials will not be the same because the shape of the plasma dilution curve obtained with the various types of thromboplastic preparations is not identical. Results obtained with Solu-Plastin® cannot be referred to a plasma dilution curve set up with rabbit brain thromboplastin or with Simplastin® or vice versa. Above all things, do not use viper venom for prothrombin time determinations in the control of anticoagulant therapy! The use of this material has been condemned by all investigators in the field as it gives completely erroneous results with dicumarolized plasmas. The whole mechanism of fibrin formation under the influence of non-mammalian thromboplastic materials, such as cobra venom, is different than the normal coagulation process, and X-ray absorption data indicate that the fibrin formed is not the same chemical entity as the fibrin formed in the normal process.

Another point which should be made is that there is no such thing as "a normal prothrombin time." Prothrombin time values will vary around a mean in the same manner as blood sugar values or basal metabolic rate. Aggeler and Lucia[16] reported an analysis of prothrombin determinations made on 64 "normal" individuals. About 43% of the determinations fell in that area above 100% activity, about 27% in the range between 100% and 90%, 19% between 90% and 80%, and 11% of the determinations between 80% and 70%. It must be remembered, when we consider the shape of the prothrombin dilutions curve, that a variation of 110% to 70% may represent a difference of only about 3 seconds in prothrombin time, and of course in this region of comparatively low sensitivity experimental error will have a greater effect upon the results. Fortunately, as far as the control of anticoagulant therapy is concerned, this region of the curve is of relatively little importance and the sensitivity of the test increases as the prothrombin activity decreases.

Some mention should be made concerning the use of diluted

plasmas for the performing of prothrombin times in anticoagulant therapy. The great majority of laboratories are using undiluted plasma, and the use of saline diluted plasma seems to product variation, particularly in the determination of the end point, which more than offsets any theoretical accuracy which might be gained in the dilution of the plasma. In the use of the prothrombin time determination as a diagnostic aid in the determination of the presence of liver disease in which the decrease in prothrombin activity may be slight, the use of diluted plasmas may be of some benefit in determining whether or not the patient has a prothrombin activity bordering on the pathological range. If the plasma is to be diluted, prothrombin-free plasma would seem to be the diluent of choice.

Various hospitals have been using the bedside test in the control of anticoagulant therapy with good results. Unfortunately, there has been a lack of standardization in the various methods—e.g.—whether or not excess calcium is added to the reaction mixture, the ratio of the amount of blood to thromboplastin and calcium, etc. There is no doubt that from the standpoint of accurate results, the regular one-stage method is to be preferred, but from the practical standpoint in the control of anticoagulant therapy, where slight variations are of relatively little importance, it would seem that the bedside test performed by a laboratory familiar with its use is sufficiently accurate for the safe control of anticoagulant therapy. That the times obtained by the bedside test as compared with those obtained by the one-stage method are not in absolute agreement is not at all surprising when we consider the difference in relative concentrations of reacting constituents under the conditions of the two tests. One probably should not take the time obtained by a bedside test, refer it to a plasma dilution curve obtained by the one-stage method, and calculate prothrombin activity on that basis. The bedside test is much more a "rule of the thumb" type of determination, and where a laboratory has had sufficient experience with the test, the results seem adequate.

Lewis, Munro, and Munro[14] compared the one-stage and bedside methods for prothrombin determinations in dicumarolized patients. At that time, they reported rather close correlation between the times obtained by the two methods. A continuation of their work indicated some variaion in this close correlation with certain lots of thromboplastin. In their most recent report[15] better correlation was obtained, and it was stated that "it would appear that a carefully controlled bedside test probably offers fewer hazards in the control of dicumarol therapy than does the one-stage test."

One other point which should be emphasized is the unques-

tioned value of keeping an accurate record of the daily shift in prothrombin activity. Prothrombin activity determinations are very similar to a reading for barometric pressure in foretelling the weather. No meteorologist could foretell the weather from a single barometric pressure reading. He wants to know whether or not the pressure has fallen or risen, and how rapidly, over the previously preceding interval of time. The same information is necessary for the intelligent administration of an anticoagulant. Is the prothrombin activity falling, or is it rising? It is easy to illustrate the importance of this by assuming a prothrombin activity determination of 20%. If the previous day's prothrombin had given a figure of 30%, additional anticoagulant would undoubtedly not be given as the next day's reading would be expected to be even lower. If, however, the previous day's reading had been 10%, the clinician might debate whether or not some anticoagulant should be given inasmuch as the next day's prothrombin activity might be above the therapeutically desired level. By studying successive day's prothrombin time determinations on a patient receiving an anticoagulant, it is also possible to determine the individual reactivity of that particular patient. Successive day's prothrombin activity determinations should ideally be charted the same way that a temperature chart is kept so that the rises and falls in activity can be seen. Convenient charts for this purpose are supplied by several pharmaceutical houses.

Extreme care in the cleaning of all glassware used in prothrombin determinations is essential. A recent episode of erratic results in a laboratory of a large New York City hospital was traced to inadequate rinsing of glassware washed in an ionic detergent. This point was also stressed in a note appearing in the February 15th, 1952, issue of *Modern Medicine*, page 84. All glassware washed in ionic detergents should be rinsed in dilute acid, followed by several rinses with tap and distilled water.

No attempt has been made, within the limited scope of this discussion, to examine other tests related to blood coagulation phenomena, such as for accelerator globulin, for other potentiating factors, nor the prothrombin consumption test. All of the cautions as to meticulous care and rigid control of techniques and reagents apply equally to all such laboratory procedures.

Summary

Unfortunately the test which we call the "one-stage prothrombin time test" does not measure prothrombin, but is a summation of all variables entering into the clotting potential of the blood. It is, however, sufficiently accurate for the laboratory control of anticoagulant therapy.

If reproducible and accurate results are to be obtained, there

must be rigorous control of the various factors which are known to alter the results obtained by the one-stage method. While reproducible results may possibly be obtained by making the same mistake every time, proper technique is the better insurance.

It should be the responsibility of the laboratory to see that the clinicians using its facilities understand the meaning and limitations of prothrombin time determinations. A short laboratory demonstration at one of the hospital staff meetings, or a joint session of the local technologists society with the local medical group offer excellent opportunities to point out that in laboratory procedures involving blood coagulation phenomena two and two may not always equal exactly four—through no fault of the laboratory.

With the availability of carefully standardized commercial thromboplastin preparations, giving reproducible results with a minimum of manipulation, there seems to be little reason for the average laboratory to go through the time consuming process of preparing and standardizing its own thromboplastin.

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EFFECT OF HOSPITAL STAFF-TECHNOLOGIST RELATIONS ON PATIENT SERVICE*

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Introduction

In regard to the activities of fellow medical technologists a prominent active member of the American Society of Medical Technologists has stated editorially earlier this year: "No world-shaking discoveries need be made—the practical, everyday solutions to small but recurring problems are of greater significance to us as a group."[1] This has encouraged us to launch upon a report growing out of the clinical observations of the past six years. The urgent need for recruits in the field of medical technology has been fully surveyed by members and patrons of this society over this period of time. Steps have been taken to fill the requests for well qualified medical technologists. Noteworthy progress has been made in establishing additional schools for their adequate training. The demands in various phases of medicine, from the research laboratory to the doctor's office, is still greater than the supply remaining after losses for various reasons are accounted for. That the greatest possible benefits may be obtained in these limited circumstances we recommend that the efficiency of the technologists available be enhanced where feasible by proper co-ordination of their tasks with those of the allied medical services, especially on the hospital front. This necessitates a functioning human relations program.

Review of Literature

The most recent census of hospital service[2] available at this time lists under expansion of educational services a steady growth in the number of approved schools of medical technology as well as a proportionate increase in the number of graduates. Despite this encouraging trend an educational authority[3] reminds us that an acute shortage of medical technologists re-

* Read before ASMT Convention, June, 1953, Louisville, Kentucky.

mains to be alleviated. Where inadequacy in personnel exists, we are admonished by another authority that "inefficiency reigns" and "the patient is the victim."[4] Later studies published assure us: "There is no laboratory which can properly function unless it is organized for efficient service. Such organizing connotes supervision of the entire unit by a person who is capable, professionally and technically, by virtue of personality, education, and experience, of managing the department's activities, of training and supervising all its personnel, and of co-operating with every other hospital department."[5]

Through their official publication, namely, the American Journal of Nursing, professional nurses have encouraged good relationships with all other departments of the hospital for the benefit of the patient. Cognizant of the growing importance of good human relations in all our professional endeavors the members of the Public Relations Committee of the American Society of Medical Technologists have prepared a handbook of objectives and suggestions for use of the profession. Their definition of public relations can here be reiterated: ". . . the essence of public relations (is) getting credit for doing a good job."[6] If the patients, who constitute a real public, can experience the effect of efficient, courteous, intelligent, devoted service the rest will follow. Obviously, such service will not be experienced unless an organization is capable of maintaining the morale of its agents.

Method

In general, the solution of the problem of maintaining morale means that those in charge know how to establish and uphold a system which not only operates in a manner conducive to their own ends but, at the same time, provides a certain measure of satisfaction for its personnel. This is to say that the system must supply the kind of satisfactions which enable the individual to identify himself with the organization, to feel that he wins or fails with it. Only then will the individual be induced to make the sacrifices required to develop and maintain the inter- and intra-departmental co-operation basic to good patient service.

Although good human relations are so essential if a service institution such as the hospital is to attain its end, namely, the welfare of the patient, it is necessary to keep in mind that there are no easy methods to attain them. An authority in the field of hospital service warns: "First, it is well to keep in mind when discussing human relations' programs that there are no 'systems' or 'gadgets' or 'techniques' which when applied will work automatically. The science of human relations has not developed to the stage where such knowledge is available. Whether we study business, industry, or service organizations, we discover such

great variety of structures and personalities that a 'method' which produces good human relations in one may utterly fail in another. This is not to say that we cannot learn from others, it merely underlines the fact that there are no panaceas, no sure fire programs in the field of human relations."[7] Basically, what those who would establish good human relations in the hospital must realize is the importance of giving people a sense of real participation. This sense of participation will be developed where individuals are respected as intelligent people who are sincerely trying to do a good job.

Results and Discussion

By means of the application of the best human relations system at our command—by means of the Golden Rule—our laboratory has taken the initiative in an effort to improve patient service in our rural 100-bed hospital during the time of this study. Our human relations program operated on two levels; first, between the technologists as a department and the other departments in the hospital: second, within the department itself among the various individuals operating as technologists. We shall discuss the program as it works on each level. In regard to the first level, allow us to clarify the term, Hospital Staff, as we have used it in the title of this paper. As we use it here, it includes the following: 1) Pathologist, 2) Medical Record Department, 3) Medical Staff, 4) Administration, 5) Nursing Staff, 6) Dietetic Department, and 7) X-ray Department. The opposite term, Technologist, refers to the administrative and technical laboratory department. The relations of the latter with each of the departments indicated in the above subdivisions will be discussed in turn.

A visiting pathologist is available three days a week on designated days. Patients, for frozen sections of biopsies or sternal punctures, are scheduled for examinations on these days. Preparation of histology slides, from the material selected by the pathologist at the gross examination of surgical specimens, is carried out ad interim between visits of the pathologist. The service of a pathologist to the hospital's surgical patients is a definite advantage over previous arrangements whereby surgical specimens were mailed to distant cities and too often long lapses of time necessarily intervened between surgical procedure and pathological diagnosis. On behalf of all patients, other advantages too numerous to mention here, have grown out of the relation of the pathologist to the general hospital laboratory.

The Medical Record Department prepares pathological reports in triplicate from the record transcribed by the pathologist. The original report is submitted for the laboratory file,

first copy for the patient's record and a second copy for the surgeon's office file. Other secretarial duties are wholeheartedly performed by the personnel of this department for the laboratory.

Members of the Medical Staff, approximately ten in number, largely general practitioners do not have available office technicians. The hospital laboratory has co-operated to accept their office patients as out-patient service. On house patients the laboratory carries out the routine procedures designated by the physician as his desired routine in order not to duplicate tests that have already been performed for the same individual as an office patient. This elimination of unnecessary tests economizes on the technologists' time permitting greater concentration on important techniques which will be of greater value to the patient.

The administration has co-operated with us and established written policies regarding admission of surgery patients. Patients for elective major surgery are admitted on the day preceding the scheduled surgical procedure in order to allow the laboratory department time to complete the pre-operative laboratory studies during the regular working day. The admitting officer directs patients for minor surgery to the laboratory at an early hour for routine tests before admitting to surgical service. This arrangement permits the laboratory to follow a definite schedule for preparation of surgical patients. Routine ward rounds are established for collection of blood specimens on medical and obstetrical patients. While fairly flexible, this schedule is a great advantage in assuring proper attention to the best interests and service of these patients. Appointments for blood donations can be arranged during the free time allowed by this schedule. Except for disruptions occasioned by emergency work this distribution of tasks over the laboratory day eliminates overtime duty for the Staff.

In order that the patient may receive the greatest possible benefits from the laboratory procedures carried out, the closest co-operation of the nursing personnel with the laboratory staff is at all times desirable. With full realization of this fact great reliance is placed on the nurse for adequate preparation of the patient for various laboratory procedures and for the proper collection of a variety of specimens. Mimeographed copies of detailed instructions for preparation of the patient and collection of specimens are sent to the division that requests special tests or procedures requiring elaborate preparations and exactly timed specimens.

Sister Charles Adele suggests a laboratory teaching program for student nurses in order to tie in better relations with this group, which we think, is an excellent idea but our hospital does not conduct a training school for nurses and we are dealing at

all times with professional nursing personnel. However, an educational program here remains a necessity. The staff nurses, all members of the local Registered Nurses Association, have participated in the educational program of said association. This includes lectures by technologists on subjects relating to various laboratory procedures and further explanations of the clinical significance of such procedures, and last but not least, emphasis on an interdepartmental public relations program. The gratifyingly complete co-operation which has been received from this group has enabled the hospital laboratory to continue to serve the institution and community with a minimum of technical assistants.

The dietetic department enhances the hospital's service to visiting patients by prompt attention to requests for diabetic breakfasts enabling the fasting patient to have a glucose test performed and to keep a later appointment with his physician on the same day. This service is greatly appreciated by those living at a distance from the hospital.

Consultation service between the x-ray department and the laboratory permits a battery of tests in each department to be performed on a single patient in the least possible length of time for hospital patients and with a minimum of visits from out patients for complete laboratory and x-ray service. A clear knowledge of the procedures to be carried out is necessary in order that conflicting x-ray procedures and laboratory procedures are not performed simultaneously. A mutual agreement entered into many years previous to this study continues in effect with the x-ray department whereby this division is responsible for Basal Metabolic Determinations and Electrocardiography.

The human relations program on the first level has been discussed, and now we enter the discussion of the second phase. The art of human relations is essential within the laboratory department. Close harmony between the administrative supervisor, who in the absence of the pathologist exercises delegated authority for the conduct of the laboratory, and the technical and clerical assistants must be constantly fostered. An oft quoted prominent business man has said that given an employee's physical presence ". . . you cannot buy enthusiasm; you cannot buy initiative; you cannot buy loyalty; you cannot buy the devotion of hearts, minds and souls. You have to earn these things."[8] This the laboratory supervisor or chief technologist has to do. A foremost authority on human relations as regards the laboratory supervisor is Sister Charles Adele, S.C.N. who has very fittingly said: "The administrative supervisor will find it most helpful to be democratic in her views with her personnel rather than to be autocratic."[9] The character of the laboratory service depends largely on the organization and adequate functioning

of the laboratory, as such. This is especially true in small hospitals where the laboratory department is a unit comprising the various fields of bacteriology, biochemistry, hematology, histopathology and the blood bank operating on a decentralized plan.

Summary and Conclusion

While working at times under difficult circumstances, such as facing excessive tasks created by inadequate technical help, ways and means to carry on worth-while contributions to the public must be found. The efficiency of medical technologists on the hospital front may be enhanced by active co-ordination of their tasks with those of the allied medical services. By means of an adequate public relations program—having for its norm the Golden Rule—we have presented a historical review of the accomplishments of one hospital laboratory. Technologists, in other and various fields from the research laboratory to the doctor's office, may make use of a public relations program for gratifying results. Constantly vigilant for opportunities to render the patients the highest quality of service, together with utilization of these opportunities, will lead to recognition of the importance of such service. "Now, of course, we'll probably never convince everyone that we're essential, or indispensable, or, in some cases, even important. But to the extent that we believe in ourselves and to the extent that we can convince others of the importance of our work, to that extent we shall have succeeded in our public relations program."[10]

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THE CULTIVATION OF VIRUSES: A REVIEW*

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The literature available on the general subject of the cultivation of viruses is indeed voluminous. This paper represents an attempt to review the more commonly used techniques of viral cultivation.

With the exception of a few reports, such as one by Olitsky,[1] who reported the cultivation of the virus of tobacco mosaic disease in the absence of living cells, all investigators have found that living cells are essential for viral multiplication. It is interesting to note that Mulvania[2] and Purdy[3] were unable to confirm the findings of Olitsky.

The ideal experimental system for virus cultivation should fulfill several requirements such as: must be susceptible to the virus being cultivated; should be free from infection before inoculation; should not have an immunity, either natural or acquired, against the virus inoculated; should not produce antibodies against the virus inoculated; should be of such a nature as to facilitate handling in the laboratory; and should show a definite, predictable, and reproducible change as a result of viral infection.

Four methods are in general use in virus laboratories for the cultivation of viruses. They are: cultivation in vitro with actively growing susceptible tissue; inoculation of a developing embryonated egg; inoculation of a suitable living host; and, in the case of the bacterial viruses, inoculation into a culture of susceptible living bacterial cells. Since these four methods are widely diverse, they will be considered separately.

Tissue Culture

Curiosity as to the mechanism of closure of the medullary tube led Roux,[11] in 1885, to isolate and cultivate the medullary plate of a chick embryo. This was the first explantation experiment on living organized tissue. In 1898, Ljunggren[11] kept transplants of human skin alive for weeks in ascitic fluid. By the turn of the century, many reports were published on successful work in transplanting tissue from animal to animal, and from animal to one or another form of tissue culture.

A simple method for tissue cultivation was developed by Harrison,[66] in 1907, from work on the cultivation of nerve fibers. Applications of Harrison's methods led to the successful cultivation of poliomyelitis, rabies, and vaccinia. Parker and Nye,[4]

*Ed. Note: This first part of the article TISSUE CULTURE, will be followed in September by the second part on **Cultivation of Viruses in the Embryo Egg**, and in November by the third part on **Virus Cultivation by Animal Inoculation.**

using a modification of Carrel's technique, were able to propagate the vaccinia virus in tissue culture for 132 days, and were able to show that one culture in the eleventh generation contained 51,000 times as much virus as the material inoculated. Here, for the first time, we have evidence for viral multiplication in tissue cultures. Carrel[5] a year later cultivated the Rous sarcoma virus with embyro pulp, fragments of spleen, and white blood cells. Unlimited multiplication of the virus was demonstrated. Since these two demonstrations of multiplication of viruses in vitro many different viruses have been propagated by tissue culture.

Special Equipment

In addition to the general equipment found in most biological, bacteriological, and virus laboratories, some special equipment is necessary for tissue culture. The type of equipment will vary as to the technique used and the type of virus being cultivated.

Special flasks have been designed by Carrel, made of optical quality glass with the neck at a 45 degree angle with the horizontal. This is to prevent or to reduce the possibility of air borne contamination, thereby facilitating the opening of six or eight flasks for manipulation at once. One of the commonly used flasks in the designer's laboratory is 35 mm. in diameter and 11 mm. in height. Shaw, Kingsland, and Brues[6] made flat bottles and drilled a hole in one side. A coverslip was cemented over the hole with a non-toxic cement, soluble in alcohol but not in water, after dry heat sterilization. Porter, Claude, and Fullam[7] used a culture flask with two flat parallel sides which measured 35 mm. in diameter. The tubes were blown from either soft glass or pyrex tubing measuring 1.5 by 16 cm.

A few special instruments are needed to handle the tissue. Cataract knives, straight and curved forceps, platinum needles in full length needle holders, and razor blades with suitable holders, are generally used. Special platinum spatulas are used for placing tissue fragments into and removing these fragments from culture tubes.

A tank containing oxygen, carbon dioxide, and nitrogen is necessary if the tissue cultures are to be gassed before inoculation and during growth. The gasses are mixed by filling a previously vacuum-evacuated tank with the desired percentages of these gases. The actual percentages of the gasses used vary with the nature of the tissue, the composition of the medium, and the purpose of the culture. Usually the actively growing tissue cultures are treated with this gas mixture every 24 hours to control the pH of the medium. Phenol red, used in concentrations ranging from 0.001 to 0.005 per cent, is non-toxic, and is used as an indicator.

Cleaning and Sterilization Procedures

Most laboratories use chemically clean glassware because of tissue's extreme sensitivity to toxic substances. Morton[8] suggests that all fragile glassware should be rinsed in water, placed in concentrated sulfuric acid containing 0.5% sodium nitrate and sodium chlorate, and then heated in the sulfuric acid solution to 90° C. and left in the solution at room temperature over night. After this, they are given several rinsings in tap water, followed by distilled water, then alcohol. With the flasks used for cultivation, the distilled water and alcohol steps are reversed.

The sterilization of instruments is by the usual autoclaving at 121° C. for fifteen minutes unless there are large bundles, when the time is extended to 30 minutes. Sterilization of heat-unstable compounds is done by using bacteriological filters.

Tissue Preparation

The source of the tissue used varies with the virus being cultivated and with the purpose of the culture. Because of their ease of availability, tissues of seven to nine day chick embryos are used commonly. Tissue obtained is minced most easily by placing it in the bottom of a 50 ml. centrifuge tube and subjecting it to repeated cutting. Cataract knives can be used to prepare tissue by placing the tissue on a piece of sterile glass and mincing it to the desired size. The latter method is preferred if it is necessary to obtain uniformity in tissue size.

Tissue Extract Preparation

Carrel,[9] in maintaining a tissue culture of connective tissue, found the embyro extracts had growth-promoting factors. This finding prompts the use of embryo extracts in tissue culture today. The extract is prepared from a nine to eleven day embryo. The eyes are removed and the embryo is cut into very fine pieces and then ground in a mortar. Salt solution, such as Tyrode's, is added so that a suspension is obtained with equal amounts of salt solution and tissue. The suspension is incubated at 37° C. for one-half hour and then centrifuged. The supernatant fluid, which is the tissue extract, is decanted and refrigerated until used.

There have been a number of modifications of the mortar and pestle. Ten Broek[10] developed a pyrex glass grinder. The outer tube of this grinder has a bulb for holding the salt solution tissue suspension. At the same level as the bulb in the outer tube, there is a constriction in the inner tube to facilitate the introduction of fluids. The plunger has a T shaped handle and is of a diameter which will just fit into the outer tube. Parker[11] modified the plunger by placing pieces of glass on the base of the plunger so that they simulated teeth. This was to make the grinder more effective with fibrous tissue.

Diluting Fluids

Ringer and Phear[12] investigated the effects of various salts on tissue and found that the tissue would continue to live in vitro if infused with a fluid containing sodium, potassium, and calcium cations used in proportions similar to those found in the blood of higher animals. Locke[11] modified Ringer's solution to correspond with higher osmotic pressures. In 1910 Tyrode[66] altered the Ringer-Locke solution by adding magnesium and monobasic sodium phosphate. The formula for Tyrode's solution is as follows:

Sodium chloride	8.00 gms.
Potassium chloride	0.20 gms.
Calcium chloride	0.20 gms.
Magnesium chloride	0.10 gms.
Sodium phosphate (monobasic)	0.05 gms.
Sodium bicarbonate	1.00 gms.
Glucose	1.00 gms.

Distilled water to make 1 liter.

Tyrode's solution contains bicarbonate and therefore must be sterilized by filtration.

Simms[11] changed Tyrode's solution to adapt it especially for tissue culture for virus propagation. Simms' solution has a reduced concentration of calcium and an increased concentration of phosphate. This solution must also be sterilized by filtration because of the bicarbonate present. Simms' solution has the following composition:

Sodium chloride	8.00 gms.
Potassium chloride	0.20 gms.
Calcium chloride	0.147 gms.
Magnesium chloride	0.203 gms.
Sodium phosphate (dibasic)	0.213 gms.
Sodium bicarbonate	1.01 gms.

Distilled water to make 1 liter.

Serum and Plasma Preparation

Fowl plasma is preferred for tissue culture because of its firm fibrin clot, transparency, and resistance to lytic enzymes. Plasma is obtained by bleeding healthy fasting fowl from the wing, carotid artery, or heart, depending on the amount of blood needed. A 1-500 dilution of heparin can be added in the ratio of 1 part to 9 parts of blood to prevent clotting, or the blood may be collected in paraffin tubes. Porter and Hawn[13] have shown that purified bovine plasma may be used as a substrate for tissue culture. Heatherington and Craig[14] found that plasma and chick embryo extract were not adequate for cultivation of mammalian

tissue and suggested the addition of either homologous or heterologous serum to the plasma and embryo extract mixture. Hetherington later found that serum, lyophilized by a method outlined by Flossdorf and Mudd[15] with embryo juice and plasma, formed excellent media for mouse cardiac tissue.

Serum is prepared from plasma or from whole blood by breaking the fibrin clot and centrifugation of the blood. Care must be taken not to hemolyze the cells present. Lindberg[11] devised a double centrifuge tube to separate serum from plasma. The outer chamber of the brass tube has a constriction of approximately two-thirds of the distance from top to bottom. An inner tube with several one-half millimeter holes drilled in the bottom rests on the constriction of the outer tube. The bottom of the inner tube is covered with a layer 5 to 10 mm. thick of quartz sand, and a layer of glass beads is placed over this. The beads cut the fibrin into small fragments and the sand prevents these fragments from passing through the holes of the inner tube. The serum is collected in the outer tube. Plasma centrifuged in this type of tube will yield over 95 per cent of its volume in serum. Homologous serum is used for tissue culture whenever possible.

Methods of Tissue Culture Involving the Use of Fluid Substrates

Maitland Medium. For growing vaccinia virus, Maitland and Maitland[16] used a medium consisting of fowl kidney in Tyrode's solution with homologous serum. This was a modification of a medium proposed by Carrel and Rivers.[17] Maitland and Maitland considered the cells of the kidney used dead, and so interpreted the resulting multiplication of virus as cultivation without tissue culture. Later workers,[18] in response to the claim of Maitland and Maitland, and of Fornet, for vaccinia multiplication without tissue culture, found that there was cell growth in the medium. The technique for preparing and testing cultures of this type has been described by Rivers, Haagen, and Muckenfuss.[18]

Maitland's medium was altered by Muckenfuss and Rivers[19] by substituting rabbit testes and serum for chicken kidneys and serum.

Simms and Sanders[66] reported the use of a serum ultrafiltrate in place of the crude serum. Cultures were incubated at lower temperatures than those of Maitland. Several viruses uncultivatable on the latter's medium are cultivatable with this modification.

Plotz Medium. Plotz[20] developed a medium for virus cultivation essentially the same as that of Maitland with the exception of the addition of a few drops of chicken plasma. The plasma forms a network of fibrin around the tissue present, giving it a tendency to float. Yields of virus are greater, due possibly to the increased cellular multiplication.

Rivers' Medium. Andrews,[21] working with Virus III, found that it could be propagated in rabbit testicular tissue suspended in Tyrode's solution. Li and Rivers[22] simplified this technique by substituting minced chick embryo for the rabbit testicular tissue. A neurovaccinia strain was used which apparently lost none of its essential characteristics in this medium. Since the original work with this medium, many viruses have been propagated by this simple inexpensive technique.

Methods Involving the Use of a Solid Substrate

Plasma or Hanging Drop. Harrison[66] devised this type of culture to observe a living developing nerve fiber. Tissue fragments are cut to about 1 mm. in greatest dimension, using care not to get fragments any larger than this as necrosis of the center will result. Tiny fragments are undesirable as cellular growth will be scanty. Optimum tissue size is correlated with optimum virus yields.

If normal tissue is used for virus cultivation, it is first immersed in a solution containing the virus. Infected tissue may also be cultivated in this manner but needs no further inoculation. Equal parts of a balanced saline solution, such as Tyrode's, and plasma are placed over the tissue which is on a cover slip. When the plasma coagulates the cover slip is inverted over a well slide and the cover slip is sealed to the slide with paraffin to make an airtight seal. Incubation temperatures depend on the type of virus being cultivated but is usually at 37° C., the optimum temperature for the tissue. If the virus is the limiting factor, so far as temperature is concerned, then incubation may be at 32 to 35° C.

Growth of the tissue can be stimulated by adding tissue extract to the plasma and Tyrode's solution mixture. If used, tissue extract is added to the Tyrode's solution in the ratio of one part to one part.

This method adapts itself well to examination during growth with the oil immersion objective of a microscope. Tissue proliferation can be watched with ease. The culture can be opened for explantation for subsequent cultures, or for fixation and staining.

Although simple, this method has severe limitations. If actively growing cells are used, they must be subcultured every two or three days. This is necessitated by the small amount of nutrients available to the tissue. The composition of the medium cannot be altered at will. Changes in the medium as a result of cell growth cannot be determined. Small quantities of tissue must be used and, as a result, virus yield is small.

Carrel's Flask Cultures. Carrel,[5] in an attempt to overcome the shortcomings of Harrison's method for tissue culture, designed a special flask for tissue culture. He used fragments of tissue, plasma, nutrient fluid, and an overlying gas mixture. The gas facilitated either a desired change in pH or holding the pH at a certain level. The tissue is placed on one wall of the flask and a drop of plasma is placed over it. The plasma coagulates and holds the tissue in place. Nutrient fluids can be changed without disturbing the tissue.

Carrel's method, although excellent, is not in common use in virology laboratories as it is more complex than necessary for viral cultivation. The value of this method lies in the field of physiology where it adapts itself well to the study of the metabolism of the cell. Carrel has pioneered the cultivation of pure cultures of cells[23, 24] with this technique. It is excellent for this type of study and may find favor with virologists to solve problems such as the effect of a virus on a special type of cell.

Roller Tube Culture. Carrel[25] in 1913 suggested the use of the roller tube type of tissue culture. It was used by Lowenstadt[26] in 1925 and further modified by Gey,[27] Gey and Gey,[28] and Lewis.[29]

Plasma is distributed evenly over the inner surface of the desired size test tube. Infected tissue fragments are placed in the plasma film and are held by the coagulation of the plasma. As the plasma coagulates the bits of tissue can be redistributed if necessary. Excess plasma is removed after the tubes have been standing 10 to 15 minutes. Nutrient fluid, such as Tyrode's or Simms' solution plus embryonic extract and serum, is added and the tube is gently rotated to insure complete wetting of the tissue embedded in the plasma. The tube is then sealed with a rubber stopper and placed in a specially designed rack. Fluids and gasses may be replaced in this type of culture when desired.

The rack is cylindrical in shape, with its axis mounted horizontally. To it is attached a motor which will, through suitable reduction gears, rotate the rack from eight to ten times each hour. The apparatus is kept in an incubator room at 37° C. or lower, again according to the type of virus being cultivated.

The chief advantage of this method over the other types of tissue culture is that it gives the cells a greater opportunity to carry on their respiratory processes since they are alternately exposed to the fluid and gaseous content of the system. Also, it is thought that harmful metabolic products and nutritive substances are more evenly distributed, thus promoting better cellular growth and subsequently greater viral production.

The nutrient fluid is usually changed each day. At intervals of one week the plasma is checked to see if it needs patching. This is done by draining the tube of nutrient solution and adding

plasma as was done when the culture was originally assembled. The clotting activity of the plasma may be hastened by the addition of a few drops of embryo extract.

The roller tube technique, although not as simple as others, is not complex. Viruses have survived longer with this type of culture than with other methods of tissue culture, and greater yields of viruses have been obtained.

Robbins, Enders, Weller and Florentino[30] were able to cultivate the three strains of poliomyelitis with this method. Recently, Melnick[31] working with the same viruses has found that in this type culture equally good multiplication took place in tubes which were not rotated as did in tubes which were rotated in the conventional manner.

Zinsser's Agar Slant Cultures. Zinsser and Schoenbach,[32] and Zinsser, Fitzpatrick, and Wei,[33] in an attempt to obtain large amounts of virulent rickettsia for vaccine production, developed an agar slant method of tissue culture which is adaptable to virus cultivation. These workers added isotonic agar, partially buffered and adjusted by adding Tyrode's solution and serum, to a medium essentially that of Maitland and Maitland.[16] Metabolic wastes are taken away from the tissue and nutritive substances are supplied by diffusion through the agar. Cheever[34] cultivated *Herpes febrilis* with this method and found multiplication of 10,000 times in 11 passages over a period of six weeks. Later he found that agar slant cultures as old as 17 days will permit the survival of inoculated *Herpes simplex* virus for at least 96 hours, and that the virus may be propagated indefinitely with fresh agar slants. Kurotchkin[35] found that vaccinia multiplied in this medium, and later Pang and Zia[66] used this technique for the propagation of St. Louis encephalitis virus.

Method of Determining Multiplication of Viruses

Perhaps the most accurate method of detecting viral multiplication was suggested by Hallauer.[36] Two sets of cultures are made in exactly the same manner, and one is incubated and the other set refrigerated. At intervals, determined by the type of virus cultivated, the contents of an incubated and a similar refrigerated tube are emptied, ground, and centrifuged. The supernatant fluid is decanted and dilutions are made. Portions of each dilution are inoculated into susceptible animals. The LD_{50} is determined and compared with that of the culture which was refrigerated. Multiplication is considered to have taken place when there is a significant increase in LD_{50}. If there is a question of the sterility of the tissue used, a third set is prepared but not inoculated with virus.

If the virus is capable of agglutinating red blood cells, multiplication can be determined most easily by a comparison of the

hemagglutinating ability of the experimental and control series.

Multiplication can be demonstrated without further testing if the virus is capable of killing the tissue culture cells. A quantitative relationship can be established by the dilution of a virus suspension of unknown concentration and subsequent inoculation of tissue cultures.

Recently Backus and Williams[37] have found that a total count can be determined by spraying a virus-poly-styrene latex suspension of known dilution on a colloidian membrane. The number of virus particles seen with the electron microscope as compared with the number of reference particles will give the total count of virus particles present. Although there is no way of separating the infective from the non-infective particles, this method could be used as an indicator of viral multiplication.

Aseptic technique is used throughout tissue culture work. Antibiotics such as penicillin and streptomycin can be used to inhibit bacterial growth without viral inhibition.

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BOOK REVIEWS

AMERICAN POCKET MEDICAL DICTIONARY: 19th Ed. Philadelphia & London. W. B. Saunders Company. 639 pages. 1953. Plain $3.25. With Thumb-Index $3.75.

Small, convenient, and handy in size is this complete listing of the terms used in medicine, nursing, pharmacy, dentistry, veterinary science, and allied biological sciences.

A HANDBOOK OF COLORIMETRIC CHEMICAL ANALYTICAL METHODS FOR INDUSTRIAL, RESEARCH AND CLINICAL LABORATORIES. The Tintometer Limited, Salisbury, England. 119 pages. Developed for use with the Lovibund Comparator.

This handbook contains techniques based on accurate color comparison whether or not the Lovibund colorimeter and discs are used. Developed from a loose-leaf form, it still allows for additional pages to be inserted as new or improved techniques may be added.

RAPID CHEMICAL MICROMETHODS FOR THE ANALYTIC FRACTIONATION OF SERUM PROTEINS. William Q. Wolfson, M.D., Rockham Arthritis Research Unit, University of Michigan Medical School, Ann Arbor, Michigan, and Clarence Cohn, M.D., Department of Biochemistry, Medical Research Institute, Michael Reese Hospital, Chicago, Illinois. The Overbeck Company. 1951. 15 pages. $1.00.

Beside a review of other simple procedures in determining abnormal values, this booklet describes simple, rapid methods of determining the major serum protein fractions.

THE SANDOZ ATLAS OF HEMATOLOGY. 2nd Ed. Sandoz Blood Atlas, 68 Charlton St., New York 14, N. Y. Sandoz Ltd., Basle, Switzerland. 1952. 91 pages. 12 tables. Illustrated section, 44 plates in color. $7.00.

This atlas is made up in loose-leaf notebook form allowing for expansion and revision. Part I. General Considerations "is devoted to a brief discussion of the terminology and classification of the blood corpuscles, followed by a description of their formation, reproduction, maturation, functions, and destruction, both under normal and abnormal conditions," followed by "technique of Blood and Bone Marrow examination."

Part II goes into the morphology of the blood cells, through a discussion of specific differential characteristics, through each system separately. The blood parasites are considered in this section.

Part III is composed of illustrations with explanatory notes. An unusual feature of this section is the series of plates on non-haemopoietic elements found in the bone marrow, as well as disintegrating forms and artefacts. There is a very complete list of textbooks and manuals of hematology,

as well as a list of references to the publications dealing with the cases illustrated in the plates.

This material could well be utilized in any clinical laboratory.

HISTOCHEMISTRY, THEORETICAL AND APPLIED A. G. Everson Pearse, M.A., M.D., Cambridge, D. C. P. London, Postgraduate Medical School (University of London) Little, Brown & Company, Boston. 1953. 530 pages. 108 illustrations. 25 tables in main text. Appendix for each chapter where applicable. $12.00.

Of primary interest to the histologist engaged in special staining techniques, this volume will be of value to the routine worker as well. The first chapters deal with the fundamentals of fixation, stains, and preparation of the different tissues. The appendices go into the specific techniques; preparation of reagents, methods, and results.

With these basic details on the relation of chemistry to histopathology, there should be a great opportunity to improve the technical application.

CIBA FOUNDATION COLLOQUIA ON ENDOCRINOLOGY. Vol. V. Bioassay of Anterior Pituitary and Adrenocortica l Hormones. General Editor G. E. W. Wolstenholme, O.B.E., M.A., M.B., B.Ch., of the Ciba Foundation, assisted by Margaret P. Cameron, M.A., A.B.L.S. Little, Brown & Company, Boston. 1953. 228 pages. 53 illustrations. $6.00.

This is the report of a conference devoted to the consideration of assays of the harmones of the anterior pituitary and the adrenal cortex. Contributors are Albert Segaloff, M. L. Dedman, A. Stuart Mason, P. Morris, C.J.O.R. Morris, I. C. Gilliland, T. Russell Fraser, A. C. Crooke, Joyce D. Matthews, R. Moricard, W. R. Buff, J. A. Loraine, E. B. Adtwood, Pamela M. Clarke, S. J. Folley, C. H. Li, E. Reid, M. P. Stark-Dunne, G. A. H. Buttle, C. H. Gray, D. M. V. Parrott, D. H. Nelson, Dwight J. Ingle, Martha Vogt, J. A. Nissin, I. E. Bush, and J. H. Gaddum.

A CURRICULUM FOR SCHOOLS OF MEDICAL TECHNOLOGY. Ed. 3. Israel Davidsohn, M.D., Professor and Chairman of Department, Chicago Medical School; Director of Laboratories, Mount Sinai Hospital; Director of Research, Mount Sinai Medical Research Foundation, Chicago, Illinois. Editor; Kurt Stern, M.D., Director, Blood Center of Mount Sinai Medical Research Foundation and Hospital, Assistant Professor of Pathology, Chicago Medical School, Chicago, Illinois. Registry of Medical Technologists of the American Society of Clinical Pathologists, Muncie, Indiana. 1953. 122 pages. Appendix.

Since most approved schools for medical technologists are already acquainted with the CURRICULUM, it will suffice to say that this edition is now available. In the introduction we find the best description of the book itself: "an outline of curriculum for the training of medical technologists." . . . "It is not a textbook." . . . "Procedures are listed" "Textbooks, monographs, and references to periodicals are recommended." "It is hoped that" . . . "this outgrowth of the authors' experiences" . . . "will prove helpful to the directors of schools of medical technology, to instructors, and to students." Chapter headings include Bibliography, Laboratory Records and Reports, Examinations, Audiovisual Teaching Aids, as well as an appendix containing various student record forms. These are beside a chapter devoted to each of the fields of medical technology.

THE GAVEL

A few weeks ago many of you journeyed to the Beautiful City of Louisville, Kentucky to attend our annual convention. There you enjoyed a fine scientific program, took part in workshops and helped make the rules which will determine the course our A. S. M. T. ship is to take in the future.

You, as representatives of your state and local societies, are participating in the national program which is necessary if we are to harvest the fruits of *good* Medical Technology and better Technologists.

Participation on a local level is very important and a precursor to a strong organization with a definite purpose in view: That of better service to the patient, our friend and neighbor. Our views may vary as day and night, however, this should not prevent us from cooperating with our fellow A. S. M. T. member to achieve our common goal of:

1. Promoting higher standards in Clinical Laboratory methods and research.
2. Elevating the status of those specializing in Medical Laboratory technique.
3. Creating mutual understanding and cooperation between the Medical Technologist and physician.
4. Promoting the mutual aid and benefits of its members.

With this goal in view may it be said of us, "Them Technologists is organized and they did things." With union of purpose and cooperation of members will we be *giving Service*, the rent we pay for living in God's world.

Mary J. Nix

AWARDS PRESENTED AT THE ANNUAL CONVENTION ASMT

Louisville, Kentucky, June, 1953

First ASMT Convention Award: to Nelma Forsythe Maclay, B.A., MT (ASCP), Chief Chemist Methodist Hospital, Indianapolis, Indiana, for paper entitled: A SPECTROPHOTOMETRIC METHOD FOR BROMSULFALEIN DETERMINATION IN JAUNDICED AND LIPEMIC SERUM.

Second ASMT Convention Award: to Hartzell G. Payne, MT (ASCP) and Philip J. Baker, Jr., Ph.D., Pharmaceutical and Chemical Research Division, Commercial Solvents Corporation, Terre Haute, Ind., for paper entitled: SULFATED DEXTRAN—BLOOD ANTICOAGULANT ACTIVITY AND TOXICITY STUDIES IN ANIMALS.

Third ASMT Convention Award: to Aileen Burge Wright, A.B., MT (ASCP), Bacteriologist, Jacksonville State Hospital, Jacksonville, Illinois, for paper entitled: THE ISOLATION AND IDENTIFICATION OF ENTERIC BACTERIA.

Hillkowitz Award: to Gordon S. Starkey, M.S., MT (ASCP), Myers Clinic Hospital, Philippi, West Virginia, for paper entitled: BLOOD VISCOSITY IN NORMAL AND DEHYDRATED PERSONS.

Registry Award: to Mildred Rose Talluto, B.A., MT (ASCP), Department of Pathology and Clinical Pathology, Rochester General Hospital, Rochester, New York, for paper entitled: A DISCUSSION ON METHODS FOR BLOOD AMYLASE DETERMINATION.

These papers will be published during the year 1953-54 in THE AMERICAN JOURNAL OF MEDICAL TECHNOLOGY.

Exhibit Awards

State: Arkansas Society of Medical Technologists

Individual: Mary Frances Gridley, Washington, D. C.

ANNOUNCEMENTS

Recruitment

THE GIRL IN THE WHITE COAT by Helen Wells, a story for the high school girl tending toward a career in medical technology is a volume soon to be announced "off the press." A later issue of this journal will carry a review.

Board of Approved Schools (joint committee A.S.C.P. and A.S.M.T.)

At the annual convention of the A.S.M.T. in Louisville, the following members were elected to serve as members of the Board of Approved Schools: Sister Mary Clare Heath, New York, for a period of three years; Mary Frances James, Alabama, two years; and Mrs. Jacqueline Bahrenburg, Washington, one year. Hereafter, one member will be selected each year to serve a three year term.

Old Journals Needed: The AMERICAN JOURNAL OF MEDICAL TECHNOLOGY will pay $1.00 each for copies of Volume 1, No. 1 of the "Bulletin of the American Society of Clinical Laboratory Technicians," November, 1934, for Volume 1, No. 1-a, January, 1935. Please notify the Executive Office, AJMT, Suite 25, Hermann Professional Bldg., Houston 25, Texas.

UNITED STATES GOVERNMENT AWARDS under the FULLBRIGHT ACT for 1954-55, for University lecturing and advanced research: Information and application forms are obtainable from CONFERENCE BOARD OF ASSOCIATED RESEARCH COUNCILS, Committee on International Exchange of Persons, 2101 Constitution Ave., Washington 25, D. C. This competition is specifically for university lecturing and postdoctoral-level research. Graduate students desiring to enroll for courses abroad or to pursue a directed program of studies at the predoctoral level should apply to their local Fullbright Adviser or directly to the Institute of International Education, 1 East 67th St., New York City, N. Y. These applications must be postmarked no later than October 15, 1953.

THE NEXT ANNUAL CONVENTION OF ASMT WILL BE HELD IN MIAMI BEACH, FLORIDA, JUNE 13-17, 1954.

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AIR FREE COLLECTION OF BLOOD BY USE OF A SILICONE STOPCOCK GREASE

By NORMAN BLEICHER, and PAUL E. JOHNSON, JR.

(The authors wish to acknowledge the helpful advice of J. Francis Dammann, Jr., M.D.; William H. Muller, Jr., M.D.; and Allan Hemingway, Ph.D., in the preparation of this manuscript.)

This work was performed in the Bay District Cardiovascular Research Laboratory of the University of California, School of Medicine, Department of Surgery, Los Angeles. Supported in part by Grant H-882 of the United States Public Health Service.

It is often necessary to obtain samples of air free blood for physical, chemical, or biologic analysis. Such samples are generally collected under oil, over mercury, in well oiled and capped syringes, or in tightly fitting syringes. They are either stored in this manner or transferred to a second container. These methods do not always prevent air contamination. Furthermore, the results of the analysis may be altered by the presence of an oil seal. In an effort to prevent these sources of error, a number of substances were tested in a search for an effective air seal and lubricant for collecting blood in syringes. A stopcock grease containing silicone proved to be the most effective.*

Preparation of the Syringes

A small amount of the stopcock grease is applied in a thin film to the plunger of a clean and dry syringe and is spread uniformly by working the plunger back and forth in the barrel. It is important that only a very thin film be applied at first, for an excess will often cause the plunger to bind permanently in the barrel. After the thin film has been applied, one should look for areas of the barrel that have not been coated. To reach these, a small amount of the stopcock grease is applied with a gauze applicator in an even thin film to the ungreased areas. Any excess grease around the lip of the barrel or on the plunger is removed. The plunger is replaced in the barrel and worked back and forth to smooth out the film. The appearance of the syringe should be similar to that of a properly greased glass stockcock. The syringes may be sterilized by autoclaving. The barrel is separated from the plunger, wrapped in gauze, and secured with a loose rubber band. Before use, the plunger is lightly wiped off with the gauze.

The syringes must be completely cleaned after each usage. They are flushed with water to clean off remaining blood, and all traces of the stopcock grease are removed by thoroughly rinsing the syringes with kerosene and cleaning with a solution of 10 Gm of sodium hydroxide and 5 Gm. of Borax in 100 cc. of water. This solution may be reused.[1]

Discussion

A thin film of stopcock grease between the barrel and plunger gives an excellent air-tight seal, and considerable negative pressure can be exerted in the syringe to aid in the withdrawal of blood. We found no indication of hemolysis in blood that had been stored in these greased syringes for as long as seven hours at temperatures from 0.5° C. to 28° C. Blood may be collected, the syringe capped, and the sample stored in the same syringe. This eliminates the need for separate collection and storage containers. The anticoagulant is contained in the syringe.

During cardiac catheterization samples must be passed from the surgeon to the technician as carefully and rapidly as possible. Loss of blood must be avoided and the operator must not be contaminated. A syringe lubricated with this stopcock grease may be held or passed in any position

* Dow Corning Stopcock Grease, Dow Corning Corporation, Midland, Michigan.

before being capped without danger of the plunger slipping. In the subdued lighting of the x-ray room, oil droplets in an oiled syringe may be mistaken for air bubbles. This confusion is overcome by use of the grease.

For highly accurate results, blood that has been allowed to stand for any length of time before being analyzed for gas content must be vigorously shaken to insure complete mixing of cells and plasma. Tests have shown that consecutive analyses of the same sample which has been hand shaken may vary greatly. The variations decrease the longer the blood is mixed. When blood is stored under an oil layer before analysis, mixing of blood is made difficult because oil droplets may become suspended in the blood and agitation of the oil surface above the blood tends to alter the carbon dioxide content of the blood.[2, 3] We have found that the thin film of stopcock grease does not alter the gasses present in the blood.

When it is necessary to centrifuge blood to collect plasma free of air, the blood need not be removed to a separate container as is the case when methods employing oil are used, but may be centrifuged in the greased syringe. The syringe is placed plunger down and revolved in the centrifuge. The syringe is kept inverted, the cap removed, and the sample taken through a tightly fitting rubber connection between the syringe tip and the collecting vessel or pipette.

Summary

A method of using a silicone stopcock grease as an air seal for the collection, storage, and centrifuging of air free blood samples in hypodermic syringes has been presented. The advantages of this method over the more usual ones have been described.

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3. Koch, F. C., and Hanke, M. E.: Practical Methods in Biochemistry. Williams and Wilkins Co., Baltimore, 1948.

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